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Metabolism of 2,3-diaminopropionate in the rat

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Abstract. L-[U-^{14}C]-2, 3-diaminopropionate was metabolised slowly in the rat. Nearly 75% of the total radioactivity could be accounted for by respiratory CO_2 (60%) and by the concentration of the isotope in the liver and kidney (15%). The rate limiting step in its metabolism may be the activity of 2, 3-diaminopropionate: ammonia lyase in the liver. It is more readily metabolised than its 3-oxalyl derivative, which is neurotoxic.

Keywords. L-[U-^{14}C]-2, 3-diaminopropionate; metabolism; rat; respiratory CO_2; liver; neurotoxic derivatives.

1. Introduction

The lower homologues of lysine, 2,4-diaminobutyrate (2,4-A_2bu) and 2, 3-diaminopropionate (2,3-A_2Pr) occur in nature in the free form or in the combined state as derivatives or form an integral part of some polypeptide antibiotics (Vijayalakshmi *et al* 1975). 2,4-A_2bu is neurotoxic while 2,3-A_2Pr is not. The 3-oxalyl derivative of 2,3-A_2Pr, the amino acid present in the free state in the pulse *Lathyrus sativus* is a potent neurotoxin both *in vitro* and *in vivo* (O'Neal *et al* 1965; Rao *et al* 1969; Watkins *et al* 1966). We have previously reported the chemical synthesis of L-[U-^{14}C]-2,3-A_2Pr and have shown that it is rapidly metabolised in a bacterial system (pseudomonad) by an inducible, 2,3-A_2Pr ammonia lyase system to pyruvate and ammonia (Rajagopal Rao *et al* 1970, 1974).

Recent reports (Rao 1975 and personal communication) on the synthesis of specifically labelled 2,3-A_2Pr and the metabolism of its 3-oxalyl derivative prompted us to report some of our findings on the metabolism of 2,3-A_2Pr in a mammalian system.

2. Materials and methods

2.1. *Materials*

L-[U-^{14}C]-2,3- A_2Pr had a specific activity of 200 μCi/mM and was synthesised by the Hofmann degradation of L-asparagine (Rajagopal Rao *et al* 1974). Male albino

Abbreviations: 2,3-A_2Pr, 2,3-diaminopropionate; 2,4-A_2bu, 2,4-diaminobutyrate.

rats (wt range 100-200 g) from the stock colony of the Institute were used in all experiments.

2.2. *Methods*

[U-^{14}C]-2, 3-A_2Pr (20 μCi) in saline was injected intraperitoneally into rats. Respiratory CO_2 was collected in a glass metabolic unit (Weinhouse and Friedmann 1951) and various tissues were processed as described earlier (Gholson *et al* 1958). Liver and kidney slices (0·5 mm thick) were cut in a Stadie-Riggs tissue slicer and the metabolic activity was measured in a Warburg respirometer. Radioactivity was determined in a G.M. counter at about 3% efficiency for ^{14}C and in some instances independently checked with a Beckman-Scintillation Counter at about 65% efficiency for ^{14}C.

3. Results and discussion

In a typical experiment (table 1) the metabolic fate of radioactive 2,3-A_2Pr indicated that about 35% of the isotope was found in the respiratory CO_2 in the first 4 h and nearly 60% of the isotope in about 8 h. At least 75% of the total radioactivity was recovered in various fractions, the major part of which was found in metabolic CO_2. These data indicated that 2,3-A_2Pr was slowly metabolised in the rat at a rate comparable to 2,4-A_2bu (Mushawar and Koeppe 1963). Although liver and kidney

Table 1. Distribution of the radioactivity in various fractions of rat tissues and in the excretory products after administration of L-[U-^{14}C]-2, 3-diaminopropionate.

Fraction	Total activity in trichloroacetic acid supernatant (μCi)	Total activity in protein (μCi)
Liver	1·35	0·02
Kidney	0·36	0·01
Spleen	0·02	
Brain	0·05	
Gastro-intestinal tract	0·36	0·05
Serum proteins		0·01
Carcass		0·80
Urine	0·60	
Respiratory CO_2	11·80*	

*Determined as $Ba^{14}CO_3$ in a G.M. counter and also as $Na_2{}^{14}CO_3$ in a scintillation spectrometer.

A male adult albino rat weighing 105 g was injected intraperitoneally with 20 μCi of L-[U-^{14}C]-2,3- A_2Pr (sp. radioactivity 200 μCi/mM) and kept in a glass metabolic unit for 8 h. Metabolic CO_2 was determined at hourly intervals. At the end of 8 h, the rat was decapitated, tissues were quickly dissected and homogenised with 5 volumes of 10% trichloroacetic acid. Radioactivity in the supernatant and residue were then determined. The carcass free of various tissues (Gholson *et al* 1958) was processed radioactivity and determined.

slices metabolised 2,3- A_2Pr very slowly (as measured in a Warburg respirometer using [U-^{14}C]-2,3- A_2Pr), our attempts to detect 2, 3-A_2Pr ammonia lyase activity in liver or kidney extracts were unsuccessful. However, it has been shown that purified cystathionate-homoserine deaminase (EC 4.4.1.1.) had a 2,3-A_2Pr ammonia lyase activity but the rate is about 5% of that of the natural substrate homoserine (Mushawar and Koeppe 1973). Consequently, in the *in vivo* metabolism of 2, 3-A_2Pr, the action of cystathionate-homoserine deaminase on 2, 3-A_2Pr may be one of the rate limiting steps accounting for the slow release of metabolic CO_2. In contrast to this slow release, other compounds like glutamate which enter the tricarboxylic acid cycle, release CO_2 rapidly (Wilson and Koeppe 1961). The exact pathway of metabolism of 2,3-A_2Pr in the rat can perhaps be determined using specifically labelled 2,3-A_2Pr and studying the labelling pattern of pyruvate-derived amino acids and other key metabolites.

Although a transaminase acting on 3-oxalyl 2,3-A_2Pr was detected in the liver, this neurotoxic amino acid appeared to be metabolised very poorly in the rat and most of it was excreted unchanged in the urine or found in the kidney (Cheema *et al* 1971a, b). On intraperitoneal administration of the tritiated neurotoxic amino acid to young rats or intrathecal administration to adult monkeys, radioactivity could be demonstrated in a population of synaptosomes which exhibit a high affinity for glutamate uptake (Lakshmanan and Padmanaban 1977). Our results suggest that the metabolism of 2,3-A_2Pr in the rat may be different from that of 3-oxalyl 2,3-A_2Pr.

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